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Combined effect of pulse electric field and probe ultrasound technologies for obtaining phenolic compounds from orange by-product

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ABSTRACT

Orange peel by-product is a known source of bioactive compounds with several health benefits. This study focuses on optimizing the conditions for pulsed electric field (PEF) technology as a pretreatment to obtain extracts rich in phenolic compounds from the orange peel. A Box-Behnken design of 15 experiments with 3 independent factors (field energy, number of pulses and pulse width) was performed. The phenolic content was extracted by ultrasound technology and analyzed by HPLC-ESI-TOF-MS. Optimum conditions were 1.4 kV/cm and 30 pulses of 110 μs achieving a recovery of 40.8 mg/g dry weight of phenolic compounds, 27.5% higher than obtained without PEF. Besides, hesperidin and narirutin content increased by 29.4 and 38.9%, respectively. For the antioxidant assays also significative increments ($\geq$ 53%) were achieved. The combination of optimized PEF pretreatment with ultrasound extraction has let to obtain orange peel enriched extracts in polyphenols with higher antioxidant activity.

1. Introduction

Pulse electric field (PEF) is a novel non-thermal green technology, which is considered to be promising for the food industry. It consists of applicating an electric field to a food matrix for a short period of time (nanoseconds, microseconds or milliseconds) resulting in the electroporation of the cells (Arshad et al., 2021). The transmembrane potential created by the electric field causes the formation of hydrophilic pores in the cell membranes. These pores increase in size and number depending on the length of the treatment or the height of the electric field strength. Into this context, PEF can cause reversible or irreversible electroporation. The latter leads to the leakage of intracellular compounds (Chakka, Sriraksha, & Ravishankar, 2021). Its efficiency depends on the process parameters (electric field strength, number of pulses and pulse width), the food product characteristics and the processing aim (Niu et al., 2020). Thus, an adequate knowledge of these factors and their influence on PEF performance is necessary to reach optimum conditions of PEF in food. The potential applications of PEF have been extensively investigated worldwide as a dehydration pre-treatment, for extracting valuable compounds or juice, for preserving food or for food structural modifications among others (Razola-Díaz, Aznar-Ramos, et al., 2023). Previously, PEF has been applied to orange peel for increasing the efficiency of extracting soluble dietary fiber also improving its physicochemical properties (Fan, Wang, Fan, Sun, & Dong, 2022). Also, Carpentieri, Režek Jambrak, Ferrari, and Pataro (2022) used PEF of 5 kV/cm during 3 h for extracting limonene from orange peels with an increase of 33% in the yield of extraction (Carpentieri et al., 2022). PEF was used as a pre-treatment at 1.20 kV/cm and 200–600 μs followed by ultrasound technology by Mello, Fontana, Mulet, Corrêa, and Cárcel (2021) for improving the drying of orange peel, shortening the drying process and preserving bioactive compounds (Mello et al., 2021). However, few research have been done using PEF for extracting polyphenols from orange wastes (Athanasiadis et al., 2022; El Kantar et al., 2018; Luengo, Álvarez, & Raso, 2013).

The combination of pulse electric field as a pre-treatment with other technologies for extracting bioactive compounds has been recently reported in other matrices such as combined with ultrasound assisted-extraction in leaves of *Aesculus carnea* (Ntourtoglou, Drosou,

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Table 1
Box–Behnken model of the conditions of extraction for the independent factors and the experimental results obtained for the response variables evaluated expressed as mean ± standard deviation.

N°	Electric field (kV/cm)	Number of pulses	Pulse width (μs)	Sum of flavonoids (μg/g d.w.)	Sum of phenolic acids (μg/g d.w.)	Sum of phenolic compounds (μg/g d.w.)	Hesperidin (μg/g d.w.)	Narirutin (μg/g d.w.)
1	1	5	50	3294.7 ± 17.5	20019.1 ± 29.3	23313.8 ± 46.8	356.9 ± 1.3	378.9 ± 1.4
2	1.8	5	50	3657.4 ± 18.0	15950.8 ± 21.8	19608.2 ± 39.8	552.2 ± 1.9	397.7 ± 1.4
3	1	50	50	6235.2 ± 26.0	22921.0 ± 32.6	29156.2 ± 58.6	925.3 ± 3.0	874.1 ± 2.9
4	1.8	50	50	4704.5 ± 21.1	20101.9 ± 27.4	24806.4 ± 48.5	628.6 ± 2.1	549.6 ± 1.9
5	1	25	15	4728.5 ± 21.8	20992.7 ± 30.3	25721.2 ± 52.1	780.1 ± 2.6	431.9 ± 1.6
6	1.8	25	15	5460.5 ± 22.9	18292.6 ± 24.9	23753.1 ± 47.7	622.7 ± 2.0	629.1 ± 2.1
7	1	25	150	6438.7 ± 26.1	23951.5 ± 33.2	30390.2 ± 59.2	934.3 ± 3.0	826.3 ± 2.7
8	1.8	25	150	6534.5 ± 26.5	26318.9 ± 36.9	32853.4 ± 63.4	902.7 ± 2.9	629.1 ± 2.1
9	1.5	5	15	3991.0 ± 18.5	20612.7 ± 27.4	24603.7 ± 45.9	523.6 ± 1.7	386.0 ± 1.4
10	1.5	50	15	3823.8 ± 18.3	17690.0 ± 23.8	21513.8 ± 42.1	670.0 ± 2.2	460.9 ± 1.6
11	1.5	5	150	6519.1 ± 27.0	26572.2 ± 38.2	33091.3 ± 65.1	975.2 ± 3.2	828.5 ± 2.7
12	1.5	50	150	7774.6 ± 29.5	27956.4 ± 37.8	35731.0 ± 67.3	1042.1 ± 3.2	962.1 ± 3.0
13	1.5	25	50	7361.7 ± 24.8	28725.1 ± 35.0	36086.8 ± 59.7	953.0 ± 2.1	895.1 ± 2.4
14	1.5	25	50	7122.9 ± 28.0	29164.5 ± 39.8	36287.4 ± 67.8	920.0 ± 2.9	923.1 ± 2.9
15	1.5	25	50	7420.5 ± 22.8	29816.9 ± 33.0	37237.4 ± 55.7	944.8 ± 2.0	940.3 ± 2.5

Dourtoglou, et al., 2022) or grape stems (Ntourtoglou, Drosou, Dourtoglou, et al., 2022) or combination with subcritical water extraction in *Citrus unshiu* peel (Hwang, Kim, Ko, & Chung, 2021). Besides, some authors have used PEF for enhance the extraction of the valuable compounds hesperidin and narirutin from other citrus matrices with promising results (Hwang et al., 2021). There are no previous references about combining optimized PEF and ultrasound technology for extracting polyphenols, specially hesperidin and narirutin from orange wastes. These specific compounds have been reported to possess several bioactivities demonstrated in humans and animals health such as anti-cancer against lung cancer (Kamaraj, Anandakumar, Jagan, Ramakrishnan, & Devaki, 2010) and breast cancer (Choi, 2007; Choi & Kim, 2011), antiatherogenic and anti-inflammatory (Abdelaziz, Abdelazem, Hashem, & Attia, 2020; Chen, Ye, Ji, & Liu, 2010; Liu et al., 2008; Wang et al., 2020), antirheumatic (Kawaguchi, Maruyama, Kometani, & Kumazawa, 2006), cardioprotective (Elavarasan et al., 2012; Jain & Parmar, 2011; Jeon et al., 2001; Rizza et al., 2011; Wang et al., 2011) and anti-diabetic (Mahmoud, Ashour, Abdel-Moneim, & Ahmed, 2012). In fact, ultrasound assisted extraction by probe was previously optimized in orange wastes with satisfactory results compared to other conventional extraction procedures (Razola-Díaz et al., 2021). In addition, the use of probe instead of ultrasound bath have demonstrated to be better in terms of reducing the extracting times and the scalability to industrial level (Aznar-Ramos, Razola-Díaz, Verardo, & Gómez-Caravaca, 2022).

So, the aim of this study was to optimize PEF conditions (electric field strength, pulse wide and number of pulses) as a pre-treatment followed by ultrasound technology with probe in orange wastes for extracting polyphenols, focusing on hesperidin and narirutin, the orange by-product compounds that have been attributed to have the highest bioactive effect. Besides the polyphenol profile measured by HPLC-ESI-TOF-MS and the antioxidant activity measured by DPPH and ABTS were analyzed in the orange peels PEF pre-treated and in a control as reference.

2. Materials and methods

2.1. Reagents and samples

Water was purified using a Milli-Q system (Millipore, Bedford, MA, USA). Vanillic acid, chlorogenic acid, ferulic acid, quercetin, and rutin were acquired from Sigma-Aldrich (St. Louis, MO, USA). Other reagents were purchased from Merck KGaA (Darmstadt, Germany).

Orange peels (dried, by-product from juice company) (var. Navelina) were purchased from a local company, as the by-product obtained after juice production. The resulting by-product was composed by the albedo and flavedo.

2.2. Experimental design

To optimize the conditions of the PEF pre-treatment for the orange by-product an experimental design was used to reduce the number of experiments and maintaining the accuracy of the experimental hypothesis. A Box-Behnken design composed by 15 experiments with three levels (−1, 0, +1) was conducted in triplicate. The independent variables were electric field (1.0, 1.5, 1.8 kV/cm), number of pulses (5, 25, 50) and the pulse width (15, 50, 150 μs), and the responses like sum of flavonoids, sum of phenolic acids, sum of phenolic compounds, hesperidin and narirutin were taken into account. The dependent variables were adjusted to a second order polynomial model equation. An ANOVA assay to evaluate the adjustment of the model was performed including the regression coefficients, the *p*-values of the regressions and the lack of fit. Statistica 7.0 package (StatSoft, Tulsa, OK, USA) was used for the mathematical operations and simulations. Besides the optimum conditions were established using response surface methodology (RSM).

2.3. Pulse electric field treatment

PEF pre-treatments were carried out in a pilot scale system (HVP 5 PEF system, ELEA, Quakenbrück, Germany) that can reach a maximum voltage up to 20 kV. The electric field was applied to the samples in a chamber with distance between electrodes of 2 cm. For each experiment 20 g of orange peels of a size of 1 ± 0.2 cm were added to 60 g of tap water (~1 mS/cm). Previously, it was stated that there is no statistically significant differences between samples cut into different sizes from 1 to 3 cm for PEF treatments (Peiró, Luengo, Segovia, Raso, & Almajano, 2019). The pulse shape (square wave bipolar pulse) was monitored online using an oscilloscope during PEF treatment. At the end of the process, the peels were filtrated and dried at 60 °C, air flow 1.6 m/s for 315 min according to previous research (Razola-Díaz, Verardo, Gómez-Caravaca, García-Villanova, & Guerra-Hernández, 2023).

For the preliminary trials different specific energy inputs (1.5–4.3 kJ/kg), frequencies (1 and 10 Hz), and total treatment times (0–7650 μs) were tested.

For the experimental design the frequency was fixed to 1 Hz for all the trials and the experiments were carried out with the conditions (electric field strength, pulse width and number of pulses) plotted in Table 1 with specific energy input in the range of 0.1–1.9 kJ/kg.

2.4. Ultrasound assisted extraction

Orange peels PEF pre-treated or non-PEF pre-treated (control) (0.5 g)

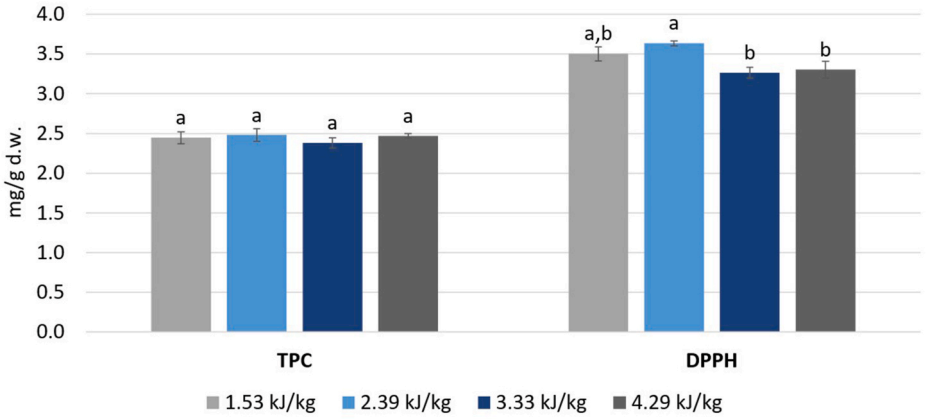

Fig. 1. Preliminary specific energy input test of PEF technology in orange peels. TPC: Total phenolic compounds. Results for TPC in mg gallic acid equivalents, and for DPPH are expressed as mg of Trolox equivalents. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

were extracted with a 45/55 ethanol/water solution (v/v) (100 mL) by a sonotrode (UP400St ultrasonic processor, Hielscher, Germany) with an amplitude of 90% for 35 min, in duplicate. Those parameters were previously optimized (Razola-Díaz et al., 2021). After the extraction, the samples were centrifuged at 3500 rpm for 15 min and the supernatant was stored at −18 °C until the analyses.

2.5. Determination of phenolic compounds by HPLC-ESI-TOF-MS

The analyses of the orange by-products were carried out in duplicate on an ACQUITY Ultra Performance LC system (Waters Corporation, Milford, MA, USA) coupled to an electrospray ionization (ESI) source operating in the negative mode and a time-of-flight (TOF) mass detector (MS) (Waters Corporation, Milford, MA, USA). The compounds of interest were separated on an ACQUITY UPLC BEH Shield RP18 column (1.7 μm, 2.1 mm × 100 mm; Waters Corporation, Milford, MA, USA) at 40 °C using a gradient previously stated by Verni et al. (Verni et al., 2020) using water containing 1% acetic acid as mobile phase A and acetonitrile as mobile phase B. Five calibration curves were made in order to quantify the phenolic compounds identified in the orange by-product. Vanillic acid, chlorogenic acid, ferulic acid, quercetin, and rutin were used as standards for quantification and their ranges, calibration curves, coefficients of determination (R^2), and limits of detection (LOD) and quantification (LOQ) are shown in Supplementary Table 1. All the compounds detected in the orange by-product samples were identified according to previous work (Razola-Díaz et al., 2021) and presented in Supplementary Table 2. Besides, a representative chromatogram is shown in Supplementary Fig. 1. The data were elaborated using MassLynx 4.1 software (Waters Corporation, Milford, MA, USA). Each extract was injected two times.

2.6. Antioxidant assays

The antioxidant activity of the orange peel extracts was determined by DPPH and ABTS methods, as described in previous research (Razola-Díaz et al., 2021; Razola-Díaz, Verardo, et al., 2023). In all the assays, the calibration curve was made of the standard Trolox, and the results were expressed in mg of Trolox equivalents (TE)/g d.w. Analyses were performed in triplicate and the measurements were carried out using an UV–visible spectrophotometer (Spectrophotometer 300 Array, UV–Vis, single beam, Shimadzu, Duisburg, Germany).

3. Results and discussion

They were carried out different steps. Firstly, they were stablished the parameter and ranges of the experimental model, and secondly the model was performed, fitted mathematically and the optimal conditions were established.

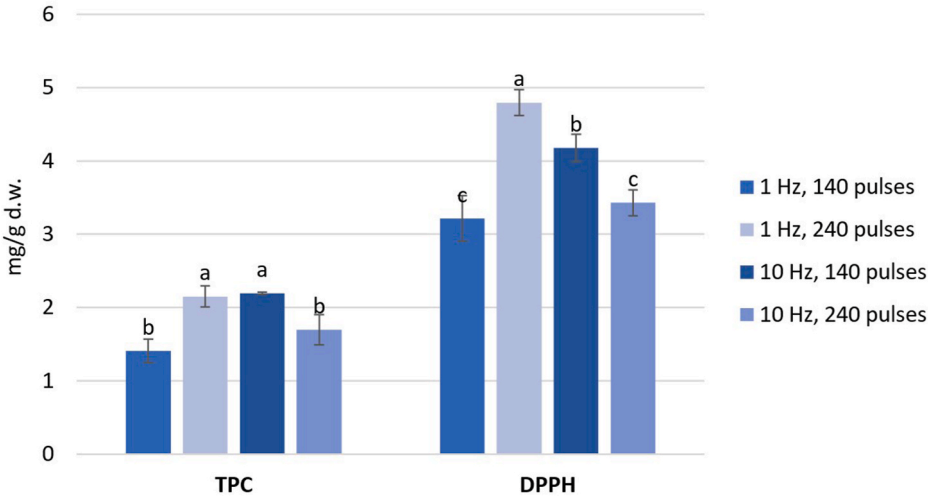

Fig. 2. Preliminary frequency test of PEF technology in orange peels. TPC: Total phenolic compounds. Results for TPC in mg gallic acid equivalents, and for DPPH are expressed as mg of Trolox equivalents. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

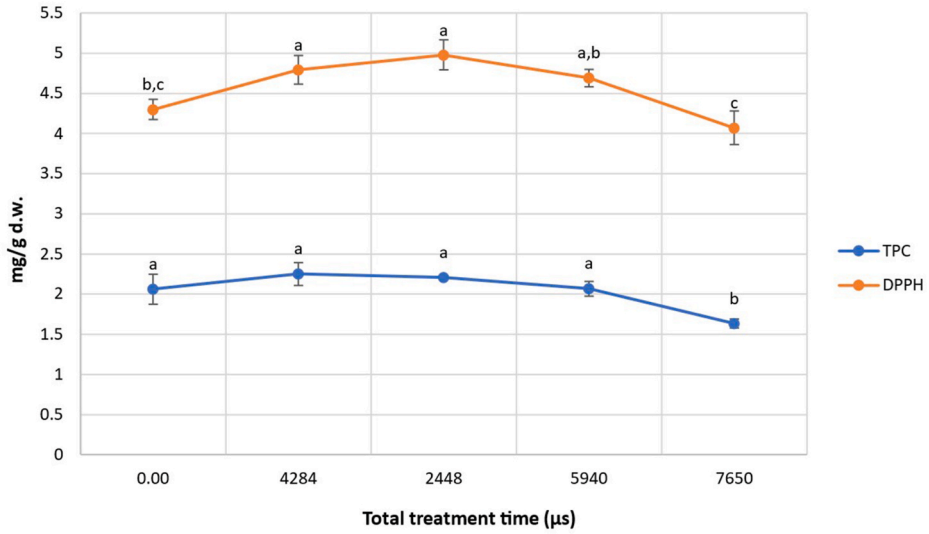

Fig. 3. Preliminary total treatment time test of PEF technology in orange peels. TPC: Total phenolic compounds. Results for TPC in mg gallic acid equivalents, and for DPPH are expressed as mg of Trolox equivalents. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.1. Establishment of the parameters of the model

Previous experiments were performed in order to establish the range of parameters for the model using as responses the total phenolic content (TPC) measured by Folin-Ciocalteu method and the antioxidant activity by DPPH technique.

In preliminary trials different specific energy (kJ/kg) were tested, and the results are presented in Fig. 1. As can be seen from the results for TPC no significative changes were observed in the range tested (1.5–4.3 kJ/kg). In the case of the antioxidant activity, a significative reduction could be shown when using a specific energy of 3.3 kJ/kg. Based on that, it was concluded that probably low specific energies could lead to better results reducing the energy consumption of the process. Thus, the voltage was adjusted to generate electric field strength in the range between 1 and 1.8 kV/cm to obtain specific energy inputs from 0.1 to 1.9 kJ/kg.

Then the specific energy input was fixed to 0.4 kJ/kg and the pulse width to 18 μs and another preliminary test were performed and are shown in Figs. 2 and 3. Fig. 2 shows the results for TPC and DPPH for different treatments varying the frequency and number of pulses. When the frequency is set to 1 Hz, a noticeable increase in the responses is observed with an increase in the number of pulses. Otherwise, at 10 Hz the opposite effect was observed when increasing the number of pulses from 140 to 240. So, the frequency was fixed at 1 Hz, reducing the number of cycles per second, to not submit to an extreme stress the orange peel cells, as the aim is to permeabilize the membrane, not to break the cells releasing the bioactive compounds directly to the water of treatment. The decision was made based on previous works (Peiró et al., 2019). Compared to using 10 Hz the treatment time is the same but increasing the operating time. Into this context the maximum operating time selected was 50 s corresponding with the number of pulses, and the lower 5 s, also determining the range of number of pulses (5–50 pulses).

Thus, another preliminary test was performed in order to select the pulse width range for the model. In Fig. 3 it can be seen the results of TPC and DPPH for different total treatment times between 0 and 7650 μs. Compared to the non-PEF-treated samples, a significant increase was observed for DPPH, while no significant changes were noted in the case of TPC when using a total treatment time of 4280 μs. However, significant ($p < 0.05$) reductions were observed at the highest tested treatment time.

So, it was concluded that total treatment times higher than 7500 μs

Table 2
Estimated regression coefficients of the adjusted second-order polynomial equation and analysis of variance (ANOVA) of the model.

Regression coefficients	Sum of flavonoids (μg/g d.w.)		Sum of phenolic acids (μg/g d.w.)		Sum of phenolic compounds (μg/g d.w.)		Hesperidin (μg/g d.w.)		Narirutin (μg/g d.w.)	
	Effect	*p* value	Effect	*p* value	Effect	*p* value	Effect	*p* value	Effect	*p* value
β_0	4577.7895	0.0003*	19174.0671	0.0002*	24073.7359	0.0002*	654.6507	0.0002*	500.1441	0.0005*
Linear										
β_1	1952.2324	0.0079*	7137.7144	0.0072*	9165.0933	0.0048*	243.5578	0.0060*	216.1110	0.0134*
β_2	2751.0616	0.0067*	6027.0306	0.0166*	8880.4979	0.0097*	339.2033	0.0052*	388.5117	0.0070*
β_3	978.4352	0.0414*	5265.7216	0.0180*	8879.4447	0.0025*	163.7251	0.0182*	115.0107	0.0607
Crossed										
β_{12}	−2191.0431	0.0105*	−3766.3866	0.0413*	−6146.0778	0.0200*	−354.0326	0.0048*	−324.0986	0.0101*
β_{13}	1160.0955	0.0338*	3931.9391	0.0356*	3267.5693	0.0295*	209.6911	0.0127*	59.3293	0.2017
β_{23}	711.3861	0.0458*	2153.4630	0.0593	3048.8536	0.0381*	−39.7549	0.1466	29.3627	0.3265
Quadratic										
β_{11}	2340.7786	0.0036*	8652.5437	0.0032*	10294.6142	0.0026*	297.7794	0.0026*	387.6758	0.0027*
β_{22}	1764.0324	0.0025*	4564.4974	0.0045*	6125.6880	0.0027*	207.6163	0.0021*	199.8211	0.0040*
β_{33}	670.8312	0.0167*	3050.0958	0.0099*	3916.7614	0.0073*	47.7907	0.0378*	150.2929	0.0071*
R^2	0.9985		0.9694		0.9783		0.9888		0.9946	
p model	0.0018*		0.0306*		0.0237*		0.0112*		0.0051*	
p lack of fit	0.4654		0.0993		0.0692		0.0874		0.2664	

*significant $p < 0.05$.

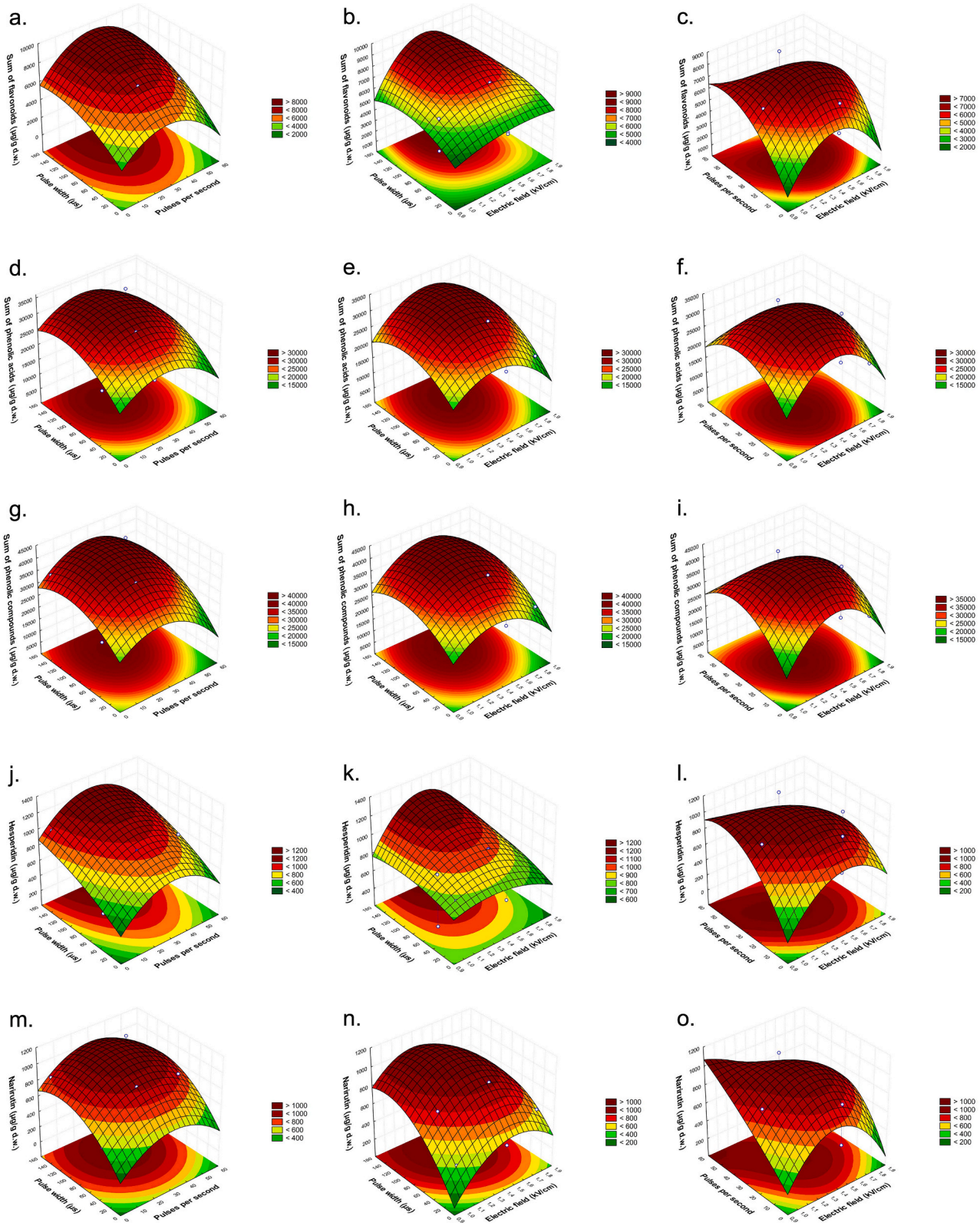

Fig. 4. Response surface plots showing combined effects of process variables for the responses sum of flavonoids (a–c), sum pf phenolic acids (*d*–f), sum of phenolic compounds (g–i), hesperidin (j–l) and narirutin (m–o).

should be avoided. A compromise between number of pulses and pulse width was done for achieving this goal, so the pulse width was established between 15 and 150 μs, leading to a minimum treatment time of 75 μs and maximum of 7500 μs.

3.2. Model fit and optimization of PEF conditions

The established ranges of the parameters electric field strength, number of pulses and pulse width were set in a Box Behnken experimental design of 15 experiments and the obtained results are shown in

Table 3
Optimal conditions selected by RSM and the model predicted values with the obtained values expressed with mean ± standard deviation.

Parameter				Optimal conditions	
Electric field (kV/cm)				1.6	
Number of pulses				30	
Pulse width (μs)				110	
	Sum of flavonoids	Sum of phenolic acids	Sum of phenolic compounds	Hesperidin	Narirutin
Predicted value (μg/g d. w.)	8964.1 ± 1232.3	32916.1 ± 4121.1	41528.8 ± 4611.1	1162.8 ± 134.0	1115.5 ± 171.5
Obtained value (μg/g d. w.)	8732.2 ± 32.4	32086.9 ± 44.3	40819.2 ± 76.7	1166.8 ± 3.5	1118.2 ± 3.6
CV (%)	1.85	1.80	1.22	0.25	0.17

CV: Coefficient of variation.

Table 1. The responses results ranged 3295–7775, 15951-29817, 19608-37237, 357–1042, and 379–962 μg/g d.w. for sum of flavonoids, sum of phenolic acids, sum of phenolic compounds, hesperidin and narirutin, respectively, all measured by HPLC-ESI-TOF-MS. The highest results of sum of flavonoids, hesperidin and narirutin were obtained in experiment number 13 where the samples were treated with 1.5 kV/cm and 50 pulses of 150 μs. Otherwise for the sum of phenolic acids and the sum of phenolic compounds, the intermedium conditions of all the parameters (1.5 kV/cm, 25 pulses of 50 μs) lead to highest recoveries. In contrast, the least favorable outcomes occurred when employing low number of pulses or a narrow pulse width. Therefore, to achieve a high recovery of the targeted compounds (hesperidin and narirutin) it is recommended to use intermediate to high values for both the number of pulses and pulse width, as indicated by these results.

These experimental data was adjusted to a second order polynomial equation and the regression coefficients are shown in Table 2. All considered response variables in the model showed positive significance for the regression (β_0), linear (β_1, β_2, β_3), and quadratic (β_{11}, β_{22}, β_{33}) terms. This indicates a significant impact of the three chosen independent factors on the modeling process.

Besides, focusing on the crossed terms, for the sum of flavonoids and the sum of phenolic compounds all the crossed terms (β_{12}, β_{13}, β_{23}) were significative. However, for the sum of flavonoids, hesperidin and narirutin the crossed term between the number of pulses and the pulse width (β_{23}) was not significant. Besides in the case of narirutin also the term β_{13} was found to not have significative effect.

The non-significant terms at $p < 0.05$ were discarded, and the model was recalculated having into account only the significant terms. The adjusted R^2 values are shown in Table 2 and all the dependent variables revealed high determination coefficients with the independent factors (0.9694–0.9985). Besides, the validity of the model was tested by ANOVA and the regression model p values were significant ($p < 0.05$) and the lack of fit non-significant ($p > 0.05$) confirming that the model can be statistically accepted.

Optimal PEF-treatment conditions were established through studying the tri-dimensional graphs presented in Fig. 4 with RSM. A compromise between all the independent factors trying to reach the maximum responses for all the dependent variables was made to evaluate the minimum values of the independent factors reducing time and energy costs. Briefly, the optimal pulse electric field conditions were an energy field strength of 1.6 kV/cm, 30 pulses and 110 μs per pulse (Table 3). The accuracy of the mathematical model was confirmed by verifying the optimal predicted values, obtaining coefficients of variation consistently lower than 5% in all cases.

Previously Athanasiadis et al. (2022) reported PEF to be a useful

Table 4
Quantification of phenolic compounds from orange peel pre-treated by PEF with the optimal conditions and no pre-treated (control) by HPLC-MS expressed as mean ± standard deviation.

Compounds	PEF pre-treated (μg/g d.w.)	Control (μg/g d.w.)
Norbergenin	5303.3 ± 2.3	4899.1 ± 2.1
Cyranoside A	< LOQ	< LOQ
2-(E)-*O*-Feruloyl-d-galactaric acid isomer a	2195.6 ± 3.5	1914.6 ± 3.0
2-(E)-*O*-Feruloyl-d-galactaric acid isomer b	1939.0 ± 3.1	1267.7 ± 2.0
2-(E)-*O*-Feruloyl-d-galactaric acid isomer c	8406.2 ± 13.0	7981.7 ± 12.4
2-(E)-*O*-Feruloyl-d-galactaric acid isomer d	4385.2 ± 6.8	2988.3 ± 4.7
Feruloyl isocitric acid isomer a	1402.2 ± 2.2	949.3 ± 1.5
Feruloyl isocitric acid isomer b	1953.7 ± 3.1	1607.2 ± 2.6
Feruloyl isocitric acid isomer c	1900.0 ± 3.0	1621.0 ± 2.6
Feruloyl isocitric acid isomer d	910.0 ± 1.5	617.3 ± 1.0
Feruloyl isocitric acid isomer e	784.1 ± 1.3	366.3 ± 0.6
Feruloyl isocitric acid isomer f	1147.5 ± 1.8	865.6 ± 1.4
Sinapic acid-*O*-glucuronide	1760.1 ± 2.8	1297.6 ± 2.1
Apigenin-di-C-hexoside isomer a	680.5 ± 2.2	408.5 ± 1.5
Rutin isomer a	373.3 ± 1.4	77.5 ± 0.5
Rutin isomer b	743.6 ± 2.4	476.4 ± 1.7
Apigenin-di-C-hexoside isomer b	46.6 ± 0.4	29.8 ± 0.4
Apigenin-di-C-hexoside isomer c	1.8 ± 0.3	< LOQ
Apigenin-di-C-hexoside isomer d	46.5 ± 0.4	2.0 ± 0.3
Prunin	176.0 ± 0.8	102.7 ± 0.6
Isorhamnetin-3-*O*-rutinoside a	260.4 ± 1.0	177.5 ± 0.8
Isorhamnetin-3-*O*-rutinoside b	159.7 ± 0.7	94.4 ± 0.6
Luteolin-C-hexoside-C-pentoside	18.0 ± 0.3	< LOQ
Alpha-glucosyl hesperidin	0.3 ± 0.3	< LOQ
Quercitrin isomer a	2.1 ± 0.3	< LOQ
Quercitrin isomer b	< LOQ	< LOQ
Eriocitrin	225.2 ± 0.9	205.9 ± 0.9
Vitexin-*O*-pentoside isomer a	115.9 ± 0.6	94.1 ± 0.6
Vitexin-*O*-pentoside isomer b	189.2 ± 0.8	152.6 ± 0.7
Naringin hydrate	166.9 ± 0.8	116.6 ± 0.6
Narirutin	1118.2 ± 3.6	683.1 ± 2.2
Hesperidin	1166.8 ± 3.5	823.1 ± 2.6
Kaempferol-3-[2″-glucosyl-6″-acetyl-galactoside] 7-glucoside isomer a	79.0 ± 0.5	46.8 ± 0.4
Kaempferol-3-[2″-glucosyl-6″-acetyl-galactoside] 7-glucoside isomer b	430.9 ± 1.5	401.3 ± 1.4
Kaempferol-3-[2″-glucosyl-6″-acetyl-galactoside] 7-glucoside isomer c	512.6 ± 1.8	475.8 ± 1.7
Kaempferol-dihexosyl acetate isomer a	156.6 ± 0.7	96.9 ± 0.6
Kaempferol-dihexosyl acetate isomer b	16.5 ± 0.3	< LOQ
Kaempferol-dihexosyl acetate isomer c	466.0 ± 1.6	314.4 ± 1.2
Didymin	244.0 ± 1.0	165.7 ± 0.8
Apigenin 7-*O*-neohesperidose isomer a	< LOQ	< LOQ
Apigenin 7-*O*-neohesperidose isomer b	< LOQ	< LOQ
Naringin 6″-malonate isomer a	< LOQ	< LOQ
Naringin 6″-malonate isomer b	5.9 ± 0.3	< LOQ
Kaempferol 3-apiosyl-(1->4)-rhamnoside-7-rhamnoside isomer a	235.9 ± 1.0	90.1 ± 0.5
Demethylnobiletin	7.3 ± 0.3	< LOQ
Isosakuranetin	4.7 ± 0.3	< LOQ
3′,4′-Didemethylnobiletin	13.1 ± 0.4	5.3 ± 0.3
Luteolin-C-hexoside-C-pentoside isomer b	15.6 ± 0.3	< LOQ
Kaempferol 3-apiosyl-(1->4)-rhamnoside-7-rhamnoside isomer b	< LOQ	< LOQ
Vitexin	< LOQ	< LOQ
Diosmetin 7-*O*-beta-d-glucopuranoside	151.6 ± 0.7	93.4 ± 0.6
Isorhamnetin 3,4′-diglucoside	22.9 ± 0.4	0.5 ± 0.1
Dimethoxyapigenin 7-glucoside	< LOQ	< LOQ
Isoquercitrin	5.6 ± 0.3	< LOQ
Kaempferol 3-rhamnoside 7-galacturonide	< LOQ	< LOQ
Isorhamnetin 3-(6‴-acetylglucosyl)(1->6)-galactoside isomer a	143.9 ± 0.7	105.3 ± 0.6
Isorhamnetin 3-(6‴-acetylglucosyl)(1->6)-galactoside isomer b	36.1 ± 0.4	< LOQ
Kaempferol 3-apiosyl-(1->2)-alpha-l-arabinofuranoside-7-rhamnoside	554.2 ± 1.9	287.8 ± 1.1
Kaempferol 3-(3″-acetyl-alpha-l-arabinopyranosyl)-(1->6)-glucoside	138.8 ± 0.7	104.6 ± 0.6
Sum of flavonoids	8732.2 ± 32.4	5631.9 ± 23.9

(*continued on next page*)

Table 4 (*continued*)

Compounds	PEF pre-treated (μg/g d.w.)	Control (μg/g d.w.)
Sum of phenolic acids	32086.9 ± 44.3	26375.6 ± 36.1
Sum of phenolic compounds	40819.2 ± 76.7	32007.5 ± 60.0

pre-treatment for extracting bioactive compounds from orange wastes. They applied a constant PEF treatment with a pulse width of 10 μs, 1000 Hz and an electric field of 1.0 kV/cm during 20 min of total treatment time (Athanasiadis et al., 2022). In this study, the total treatment time needed for reaching the highest phenolic content was 3.3×10^{-3} s, a much lower time. As reported previously the electric field needed for generating a permeabilization of the plant tissue membrane range between 0.5 and 9 kV/cm (Heinz, Toepfl, & Knorr, 2003; Puértolas, López, Condón, Raso, & Álvarez, 2009), thus, the energy field strength found here as optimum was in the range accordingly with the literature. Other by-products such as pomelo peel, Niu, Ren, Li, Zeng, and Li (2021) obtained the best narirutin extraction by using 4 kV/cm and 30 pulses (Niu et al., 2021).

3.3. Comparison of PEF pre-treated and non-pre-treated orange peel

The orange peel PEF pre-treated with the optimized conditions (1.6 kV/cm, 30 pulses and 110 μs) and extracted by ultrasound technology has been compared with the orange peel extracted by ultrasound technology without the PEF pre-treatment and all the quantified compounds are shown in Table 4. With the established PEF-pre-treatment optimal conditions an increment of 27.5% in the total phenolic compounds compared to only extracting by ultrasound technology could be achieved. Increase in the sum of flavonoids (35.5%) and phenolic acids (17.8%) were also observed. Besides, increments in the hesperidin and narirutin content of 29.4 and 38.9% were obtained, respectively. El Kantar et al. (2018) reported an enhancement of 27.3% in the polyphenol extraction from orange peels using a PEF treatment of 10 kV/cm previously to extraction. However, they did not report increase in the hesperidin content in the albedo, nor in the flavedo of the orange peels (El Kantar et al., 2018). Athanasiadis et al. (2022) reported a comparison between different pre-treatments to improve the extraction of bioactive compounds from orange peels. They also concluded that the combination between PEF + US was better than using one of the technologies alone for the extraction of phenolic compounds. For other by-product, such as grape stems, pomelo peel and onion skins, similar increases were reported. According to Ntourtoglou et al. (2022) an increase up to 35% in the polyphenol extraction with PEF as pre-treatment followed by US extraction in grape stems was found (Ntourtoglou, Drosou, Chatzimitakos, et al., 2022). Niu et al. (2021) showed a 20% increase in naringin extraction using PEF as a pre-treatment in pomelo peel, and a 70% increase in its antioxidant activity. In onion skins an increment of 33% of the quercetin content was achieved using PEF as a pre-treatment (Kim, Ko, Park, & Chung, 2022).

Moreover, the antioxidant activity of the PEF pre-treated, and the control was performed by DPPH and ABTS assays (Fig. 5). Both methods had significative strong correlation with an $r = 0.9994$. As it can be seen from the results a significative increase of 53.1 and 57.9% for DPPH and ABTS were achieved in the antioxidant activity when using the optimized PEF conditions. Luengo et al. (2013) reported yields of 51%, 94%, 148% and 192% in the antioxidant compounds in the extracts of orange peel submitted to PEF pre-treatments of 1, 3, 5 and 7 kV/cm followed by pressing extraction, compared to the extracted by pressing without pre-treatment (Luengo et al., 2013). The increase in the antioxidant activity of PEF-treated orange peel extract is not only due to the permeabilization of the membrane that leads to the leakage of more antioxidant compounds but is also influenced by structural changes of the investigated compounds as reported by Niu et al. (2021). They reported that when submitting naringin to an electric field of 6 kV/cm the microcrystalline structure was more regular and with a crystal size distribution more uniform compared to non-PEF treated and therefore having a higher antioxidant activity. On the other hand a higher electric field strength leads to irregular agglomerates which are decreasing the antioxidant activity (Niu et al., 2021). Thus, at the low electric field strength used in this work, the crystal conformation of the polyphenols might be more regular leading to an increasing in the antioxidant activity of the orange peel extracts.

Additional studies are needed to delve into the physiological and structural changes induced by PEF for a deeper understanding of the observed outcomes. Findings are limited to the selected experimental conditions and orange peel samples; generalizing to other conditions or waste materials may need further validation. Moreover, the feasibility of the methods in fresh by-products could be also checked to corroborate the influence of a possible different structure on phenolic release.

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CRediT authorship contribution statement

María del Carmen Razola-Díaz: Writing – original draft, Investigation, Formal analysis, Data curation. **Robert Sevenich:** Writing – original draft, Investigation, Formal analysis, Data curation. **Luma Rossi Ribeiro:** Writing – review & editing, Conceptualization. **Eduardo-Jesús Guerra-Hernández:** Writing – review & editing, Supervision, Funding acquisition. **Oliver Schlüter:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Vito**

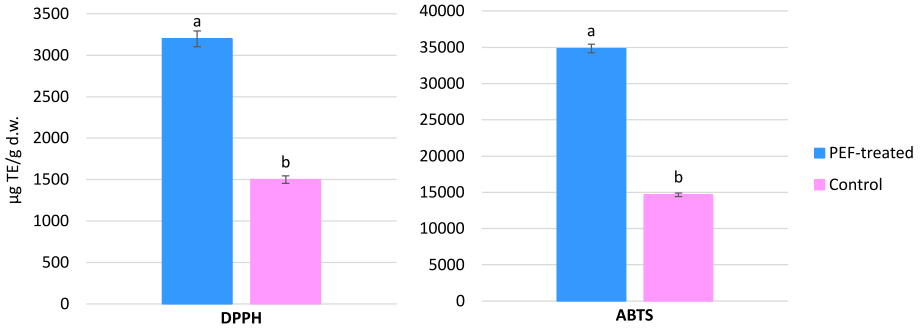

Fig. 5. Antioxidant activity measured by DPPH and ABTS of the optimum PEF-treated orange peel extract and the control non PEF-treated. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Verardo: Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2024.115950.

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